

THE RÔLE OF PROTEINS IN METABOLISM

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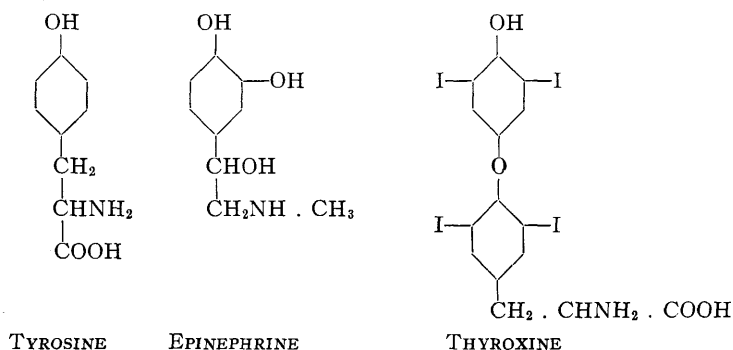
Proteins occupy a unique position in metabolism. Not only may they serve as sources of energy for the organism, but they constitute the most important raw materials out of which the complex structures of the body are built. While carbohydrates and fats are employed chiefly as fuels for the "protoplasmic fires," proteins, in the well balanced diet, serve mainly for purposes of synthesis.

Modern investigations have revealed the fact that proteins are composed of at least twenty amino acids. The amounts and methods of union of these acids vary widely in proteins of different sources. Indeed, no two proteins are exactly alike. Inasmuch as body proteins have their origin in those of the food, the latter must undergo complete disintegration before they can be utilized for synthetic purposes. This preparatory process is accomplished in the alimentary tract. At the completion of digestion the free amino acids pass unchanged into the portal circulation. Thence they are distributed throughout the organism, and serve as the substrates for the many synthetic transformations involved in the production of tissue constituents.

Very little information is available concerning the mechanism of protein anabolism. Each tissue protein is believed to be synthesized in the locality in which it is to exist. But the dominant factors which enable the cells to combine the amino acids with each other in the correct order, and in exactly the right proportions, are still beyond the realm of our comprehension. It may be shown mathematically that the number of possible isomeric proteins containing a single molecule of each of the twenty amino acids, united by the so-called peptid linkage, amounts to the incredible figure of 60×10^{20} . When one recalls that proteins are not made up of *single* molecules of the individual amino acids, but contain *many* molecules of each kind; and that several methods of union, instead of one,

may be employed in their synthesis, the number of possibilities is seen to be infinite. Thus the unerring accuracy with which specific proteins are synthesized out of the varying mixture of amino acids circulating in the blood is one of the most astounding attributes of living things.

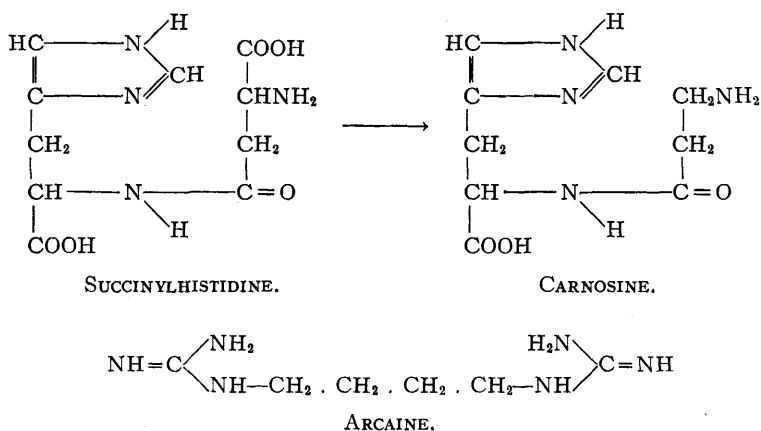
But proteins are not the sole tissue components which originate in amino acids. Numerous non-protein nitrogenous materials arise in a like fashion. Thus, many of the hormones, and certain of the so-called "extractives" are manufactured from amino acids. Of the hormones, epinephrine and thyroxine are the best known chemically. Their structures indicate that they are probably derived from tyrosine or phenylalanine.



The mechanism of their formation, however, is still quite unknown. Diiodotyrosine, also found in the thyroid gland, may be an intermediate in thyroxine synthesis. The pancreatic hormone, insulin, appears to be a simple protein or polypeptid, and as such must be formed from the amino acids of the blood. Indeed, several amino acids have been recovered from the hydrolysate of crystalline insulin, notably, cystine, histidine, lysine, and tyrosine (du Vigneaud, Jensen, and Wintersteiner, 1927-28). Probably other internal secretions, such as those of the parathyroids, pituitary, and sex glands are derived from amino acids.

Of the extractives, carnosine, and its homologue anserine, isolated from muscle tissue by Ackermann and his associates (1929), are closely related to well known amino acids. The suggestion has been made that carnosine may be formed by the decarboxylation of the dipeptid, succinylhistidine. The recently described extractive, arcaine, discovered by Kutscher,

Ackermann and Flössner (1931) in the muscles of the invertebrate, *Arco noae*, may have its origin in arginine. Investigations of Ackroyd and Hopkins (1916), as well as those in the author's laboratory (Rose and Cook, 1925), appear to indicate that the purines, which are so important as components of the cell nuclei, are formed, at least in part, from the histidine of the diet.



The extractive which has attracted the greatest interest, and occasioned the most controversy is creatine. This compound is the most abundant of the non-protein nitrogenous constituents of vertebrate muscles. It has acquired new importance in recent years because of its relation to muscular activity. The discovery of Fiske and Subbarow (1929) that creatine in the cells, exists for the most part in combination with phosphoric acid, opened the way for the elucidation of the extraordinarily complex process of muscular contraction. While all of the details are not yet clear, and are beyond the scope of this review, apparently the first change in the muscle, and the one which is primarily responsible for the contraction, is the hydrolysis of phosphocreatine into its components. This involves the liberation of about 11,000 calories of energy per gram-molecule of phosphocreatine decomposed (*cf.* Meyerhof and Lohmann, 1928). During the recovery or relaxation phase of muscular activity, the creatine and phosphoric acid are recombined at the expense of energy set free in the decomposition of carbohydrates or fats. The process is much more

complicated than our brief outline suggests, but the above will suffice to indicate the important role played by creatine in human economy.

The recognition of the function of creatine has served to renew interest in its origin. For many years attention was directed toward arginine as its most probable precursor. Doubtless this idea arose because arginine is the only known protein component which contains the guanidine group characteristic of creatine. Space does not permit a detailed review of the many efforts to augment the creatine content of the muscles, or the creatinine output in the excreta by the administration of excessive doses of arginine. The majority of such investigations have yielded negative results. Even the prolonged (6-8 weeks) feeding to human subjects of daily doses of the amino acid equivalent to 1 gram of creatine failed to provide any evidence for a transformation of exogenous arginine into creatine or creatinine (Hyde and Rose, 1929).

In recent years attempts have been made to correlate creatine with other amino acids. Brand and his associates (1929-32) report that the administration of glycine to patients with progressive pseudohypertrophic muscular dystrophy may induce a 40 per cent increase in creatine excretion as compared with the control level without glycine administration. Furthermore, gelatin, a protein rich in glycine, is said to greatly enlarge the creatine output; while edestin, relatively low in glycine, exerts little effect. These observations have been confirmed and extended by Thomas, Milhorat and Techner (1932), who report a remarkable improvement in the clinical symptoms of dystrophy patients following glycine feeding. The sensation of fatigue so characteristic of the disease is said to diminish, and the function of certain muscle groups improves to the extent that activities formerly impossible can now be performed. The authors conclude "that glycine has an important function in muscle physiology, and a significant role in the pathogenesis and treatment of this disease."

Unfortunately, not all investigators are in accord with the view that creatine excretion is stimulated by glycine ingestion. Thus Abderhalden and Buadze (1932) report that the oral administration of 15 grams of glycine is without influence upon the creatine-creatinine metabolism of the dog. On the other hand, they present data indicating that histidine and purines are the precursors of creatine. They believe that the

relationship is a direct one in which the imidazole group is transformed into the extractive.

It is impossible at the present time to harmonize the results of the glycine and histidine investigations. One should note, however, that the conditions employed in the two studies were radically different. In the glycine experiments, diseased human patients served as the subjects, while in the histidine investigations presumably normal animals were used. In human dystrophy cases there exists unquestionably a creatine deficiency in the muscles, which doubtless is an important factor in the loss of muscular strength. Such individuals would appear to be particularly well suited for studies of the origin of creatine. But even after taking these facts into account no satisfactory explanation for the contradictory findings is evident. At this time we are interested primarily in emphasizing the extraordinary role which the amino acids play, both in the chemical make-up and in the physiological functions of living things. Their uses in the normal individual, as well as their relation to the etiology of disease, are problems the solution of which will demand the greatest ingenuity of the investigator.

The multitude of synthetic reactions in which the amino acids participate cannot but excite one's curiosity as to the quantity of protein which is required for normal human nutrition. The controversy over the problem of the optimal protein intake, which was so actively debated for many years, is familiar to all students of metabolism. On the one hand, the Voit "standard" of 118 grams of protein per day, arrived at by a statistical study of a large number of human diets, was advocated with vigor by its proponents. They pointed out in no uncertain terms the dire consequences which would befall those who had the temerity to lower the protein intake "in the light of the accumulated evidence of the ill-effects that follow in the train of chronic underfeeding." At the other extreme, the disciples of the low protein regime were equally outspoken in their denunciation of the evils of a high protein ration. One of these investigators expressed himself as follows: "The combustion of proteid within the organism yields a solid ash which must be raked down by the liver and thrown out by the kidneys. Now when this task gets to be over-laborious, the laborers are likely to go on strike. The grate, then, is not properly raked; clinkers form, and slowly the smothered

fire glows dull and dies" (Curtis, quoted by Chittenden, 1907, p. 269). Another exclaimed: "I have already said that it would seem to be practically impossible to avoid getting protein enough! Does it not appear to be quite in the order of things that, practically, we must always get enough protein for our needs if we eat as Nature dictates? How, otherwise, could mankind have progressed so well since some hundred thousand years before Christ until A. D. 1866, in which year Voit stepped on the stage with his standard? Nature makes such careful provision in all things that there could be nothing more certain than that a means of nourishment so indispensable to all animal organisms as protein actually is must be present in such generous measure in all our food-stuffs that there is not the slightest necessity to fear that we should be unable to get an adequate supply" (Hindhede, quoted by Mendel, 1923).

In the meantime, Chittenden, in extensive experiments upon himself, his students, and others, had demonstrated that nitrogen equilibrium can be secured and maintained with a daily intake of 45 to 55 grams of protein, a quantity less than one-half that of the Voit standard. The importance of Chittenden's observations, in showing the extent to which the protein content of the diet may be reduced under suitable experimental conditions, should not be underestimated. The determination of the minimal nitrogen intake compatible with the maintenance of the *status quo* of the cells was of fundamental significance. On the other hand, it does not follow that a very low intake is, under all circumstances, necessarily the optimal. When Chittenden's studies were first undertaken in 1902 much less was known concerning the chemical composition of proteins than is recognized today. The majority of the amino acids had been discovered prior to 1900, but very limited data existed concerning their relative distribution. Consequently, there prevailed little appreciation of the fact that the nutritive value of a protein depends just as truly upon the *kind* as upon the *quantities* of its components. Under the circumstances, it is not surprising that perhaps undue emphasis was placed upon the *amount* of protein ingested, and that scant consideration was given to possible differences in nutritive *quality*. Indeed, not until the advent of the Kossel and Kutscher (1900-01) procedure for the isolation of the diamino acids, and the Fischer (1901) ester method for the separation of the monoamino acids, were reasonably adequate

tools in the hands of the biochemist for the study of the make-up of proteins. The discovery of these valuable improvements in technic marked the beginning of a new era in our knowledge, not only of the chemistry of proteins, but indirectly of their physiological value as well. It was soon seen that proteins of different sources vary enormously as regards the proportions in which their constituent amino acids occur. Frequently, one or more amino acid was found to be missing entirely. Thus gliadin of wheat proved to be deficient in lysine; zein of corn was shown to be practically devoid of lysine and tryptophane; and gelatin was seen to be lacking in tryptophane, tyrosine, cystine, valine, isoleucine, and hydroxyglutamic acid (Dakin, 1920).

The recognition of these facts naturally raised the question as to the nutritive importance of the individual amino acids. Inasmuch as all of the generally recognized amino acids occur as components of tissue proteins, obviously each must be made available, either preformed in the diet, or by synthesis in the organism from other materials. Thus protein metabolism immediately became a much more complex phenomenon than was originally supposed. Instead of being concerned with a single dietary factor, it was now seen to involve each of the so-called "Bausteine" of proteins of which eighteen had been discovered by 1912. As stated by Osborne and Mendel in 1914, "Obviously the relative values of the different proteins in nutrition are based upon their content of those special amino-acids which cannot be synthesized in the animal body and which are indispensable for certain distinct, as yet not clearly defined processes which we express as maintenance or repair." (Osborne and Mendel, 1914a). As a result of this new view-point attention was directed in several laboratories toward determining which amino acids are necessary dietary components.

Among the earlier studies of the role of the individual amino acids in nutrition those of Osborne and Mendel are of extraordinary importance. These investigators (1914a) demonstrated the indispensable nature of tryptophane and lysine by feeding diets containing zein as the sole protein. They observed that young rats upon such rations not only fail to grow but rapidly lose weight. The addition of tryptophane to the food leads to maintenance but no growth, but the inclusion of both tryptophane and lysine is followed by rapid growth. In like

manner, when gliadin of wheat serves as the sole protein of the diet growth does not occur until lysine is incorporated in the ration (Osborne and Mendel, 1914a). Unquestionably, both lysine and tryptophane are indispensable dietary components. Furthermore, it is not necessary to supply the missing amino acids in the free state. The supplementation of a zein diet with some other protein containing adequate quantities of lysine and tryptophane results immediately in growth (Osborne and Mendel, 1914b).

In a similar fashion Osborne and Mendel (1915) showed that cystine is essential. When an otherwise adequate ration carries 18 per cent of casein, young rats receiving such a food mixture grow at normal rates. When, however, the proportion of casein is progressively diminished cystine becomes the limiting factor. At a 9 per cent level, casein is incapable of inducing normal growth; but the addition of cystine renders the diet adequate, and growth promptly ensues. Confirmatory evidence for the indispensable nature of cystine has been reported from several laboratories. Johns and Finks (1920) found that the addition of cystine to diets containing phaseolin markedly improves the nutritive quality of the food. Like results were secured by Sherman and Merrill (1925) in the use of a diet of whole milk powder overdiluted with starch.

Recently, Jackson and Block (1931, 1932) have made the remarkable observation that methionine may replace at least a large part of the cystine of the diet. These results have been confirmed by Weichselbaum, Weichselbaum, and Stewart (1932). It is not yet clear whether the substitution is due to a direct transformation of methionine into cystine, or to the possibility that each of the two amino acids may perform independently certain functions in which both ordinarily participate. Nor is it yet known whether a reverse replacement of methionine by cystine can occur.

A fourth indispensable amino acid is histidine. In 1916, Ackroyd and Hopkins observed that when arginine and histidine are removed from acid-hydrolyzed casein, the resulting material is inadequate for maintenance or growth. The authors state that if either arginine or histidine is included in the ration, no loss in weight occurs, and growth may be resumed. From these results they concluded that the two amino acids are interchangeable in metabolism, but that at least one must be present in the diet. In so far as the indispensable nature of

histidine is concerned, the experiments in the writer's laboratory completely confirmed the findings of Ackroyd and Hopkins (*cf.* Rose and Cox, 1924). We were unable, however, to demonstrate an interchangeable relationship between the two amino acids. The addition of histidine to the diet invariably induced an immediate and rapid increase in weight of the animals, but the inclusion of arginine exerted no influence upon growth even when the quantity added was more than equivalent to the sum of the arginine and histidine present in casein. Confirmatory evidence in support of the essential nature of histidine was furnished by later publications from the writer's laboratory (Cox and Rose, 1926), and by Harrow and Sherwin (1926).

Thus, these four amino acids—tryptophane, lysine, cystine, and histidine—are known to be absolutely indispensable dietary components. In the absence of either nutrition fails, and eventually death results regardless of how much other food is consumed. The probability exists that either tyrosine or phenylalanine is also a required dietary component. Recently, at the University of Illinois, we have undertaken a study of the effect of combined crystallization and selective absorption as a method of removing amino acids from hydrolyzed proteins. While the investigation is not yet completed, a material has been obtained from casein which when suitably supplemented with certain purified amino acids, including tyrosine and phenylalanine, supports satisfactory growth. In the absence of tyrosine and phenylalanine, growth is impeded. At an early date we shall know whether one or both of the compounds in question must be present in the food. We are confident that at least one is indispensable. Several years ago Abderhalden (1915, 1922) presented data which he interpreted as indicating that either tyrosine or phenylalanine must be included in the food, but that the two are mutually interchangeable in metabolism. On the contrary, Totani (1916), and Lightbody and Kenyon (1928) were unable to demonstrate any relationship between the growth of rats and the tyrosine content of the diet. The conflicting results in the literature necessitated the investigations in which we are now engaged. So far our findings confirm those of Abderhalden.

In contrast to the amino acids discussed above, much evidence is available indicating that certain protein components may not be necessary. It is well known that when

benzoic acid is administered to man or to most animals it is conjugated with glycine, and is eliminated in the urine as hippuric acid. By measuring the maximum production of hippuric acid several investigators have reported that the output may carry more glycine than is found preformed in the proteins metabolized. The origin of the glycine is unknown, but it has been shown that a considerable portion of the nitrogen which in the normal metabolic processes is converted into urea, may, after excessive doses of benzoic acid, be diverted to the synthesis of hippuric acid. These findings led to the general impression that glycine may be synthesized by the organism out of ammonia and non-nitrogenous materials, or from other amino acids. As further evidence in this direction the fact has sometimes been emphasized that casein, though low in glycine, serves admirably for purposes of growth in both man and animals (Abderhalden, 1915). In like manner gliadin and zein, both of which are believed to be devoid of glycine, are made satisfactory for growth by suitable supplementation without the addition of glycine.

The evidence, however, is not all in favor of the dietary dispensability of this amino acid. Griffith (1929-30) has shown that the growth of young rats may be inhibited by the inclusion of benzoate in the diet unless glycine as such, or in the form of protein, is supplied in amounts sufficient to detoxicate the benzoate, and meet the needs of tissue synthesis. While the author believes that his data "support the idea that glycine is synthesized by animal tissues," evidently the synthesis is limited in extent. Apparently, the use of glycine for detoxication purposes may create a deficiency for the growth function unless an increased supply of the amino acid is provided. In the light of these investigations one must conclude that the prevailing idea that glycine may be formed practically *ad libitum* by the animal organism is at the present time scarcely warranted.

The relation of arginine to maintenance and growth has been the subject of several investigations. Reference has already been made to the papers of Ackroyd and Hopkins and of Rose and Cox involving the feeding of casein digests from which both arginine and histidine had been precipitated. According to Abderhalden (1922) arginine is probably indispensable in nutrition. His investigation, involving the use of mixtures of purified amino acids, is in some respects remark-

able; but owing to the difficulties experienced in the synthesis of the dietary components, the available materials were necessarily limited, and the feeding trials were few in number and of short duration. His results appear to be open to the further criticism that frequently his animals were provided with inadequate supplies of vitamins. Using an entirely different procedure, Crowdle and Sherwin (1923) report that fowls are capable of synthesizing ornithine for the detoxication of benzoic acid. Since ornithine is a component of arginine, the observation suggests that the latter also may be a synthetic product, at least in the species in question.

In view of the uncertainties inherent in the above experiments the arginine problem was attacked by a different method (Scull and Rose, 1930). This involved a comparison of the arginine intake of growing rats on an arginine-low diet and the increments in tissue arginine, in order to determine whether the latter may be accounted for by the amounts of the amino acid in the basal rations and vitamin supplement. For this purpose, hydrolyzed casein was rendered as nearly devoid of arginine as possible, and was incorporated in a diet which was administered *ad libitum*. At the beginning of the experiments, litter mates of the animals employed in the growth studies were killed and subjected to analysis *in toto* for arginine. The other members of each litter were killed and analyzed after they had received the experimental diet for a period of 64 days. In the meantime they had gained 73 to 113 grams each. Without exception the increase in tissue arginine was 2 to 3 times as large as could be accounted for by the total arginine content of the food. The findings "seem to warrant the conclusion that arginine may be synthesized by the organism of the rat, and in this species at least is not an indispensable dietary component" (Scull and Rose, 1930).

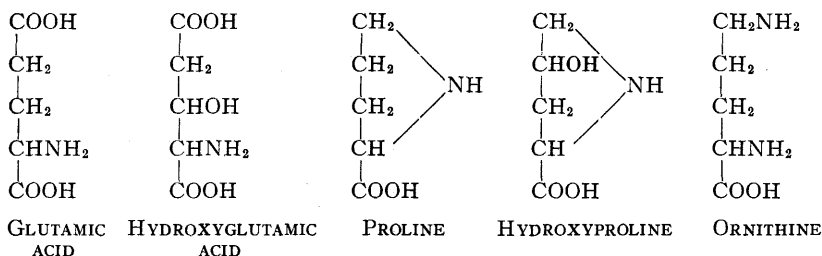
Investigations concerning the relation of certain other amino acids to nutrition have been made, but at the present time the results scarcely warrant positive conclusions. A number of years ago Abderhalden (1912) suggested that proline might be a dispensable amino acid. On the other hand, Sure (1924) is of the opinion that it is necessary. His data, however, are not very convincing. St. Julian and Rose (1932) have removed proline as completely as possible from hydrolyzed proteins by 40 extractions with hot absolute alcohol, without impairing the growth-promoting value of the resulting material. The

same investigators have precipitated the dicarboxylic amino acids, glutamic, hydroxyglutamic, and aspartic acids, without diminishing the nutritive properties of the residue. Hopkins (1916) is of the opinion that neither glutamic nor aspartic acid is indispensable. Sherwin, Wolf, and Wolf (1919) report that in the human subject glutamine may be synthesized for the purpose of detoxicating phenylacetic acid, which in man is excreted in the urine as phenacetylglutamine. Hydroxyglutamic acid is usually listed as non-essential inasmuch as edestin, which presumably is devoid of this amino acid, supports normal growth (*cf.* Osborne and Mendel, 1915; and Osborne, Leavenworth, and Nolan, 1924).

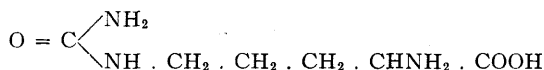
Very little information is available concerning the possible nutritive importance of hydroxyproline. Spörer and Kapfhammer (1930) call attention to the fact that several vegetable products, notably soy-bean flour, do not contain detectable amounts of this amino acid. The authors suggest that in view of the quantitative importance of soy-beans in the dietaries of oriental peoples, hydroxyproline may not be an essential constituent. More recently, Adeline (1931) has reported that rats upon diets containing 6 per cent of edestin cease growing after approximately 12 weeks, but that the addition of either proline or hydroxyproline remedies the nutritive deficiency, and induces growth. She concludes that the two compounds are interchangeable in metabolism. Unfortunately, the data are not so extensive or clear-cut as one would wish, in view of the conflicting evidence regarding the subject.

The interpretation of the results of feeding experiments in which proline, hydroxyproline, and the dicarboxylic acids are supposedly absent from the diet is rendered difficult by the lack of delicate tests for these amino acids. In such investigations one must recall that even traces of a life-essential may suffice to meet the growth requirements of the organism. It is possible that the prolines, glutamic acids, and the ornithine part of arginine may all be interchangeable in metabolism, or be capable of yielding a single essential. Thus if proline were necessary but absent from the food, one or more of the other four might be transformed into the missing amino acid, and thus prevent a dietary deficiency. The similarity in structure of the five compounds is readily seen from the accompanying formulas. Indeed, Abderhalden (1912, 1922) has frequently suggested that glutamic acid and proline may be

capable of replacing each other. We have attempted to secure evidence for such a substitution by removing all five amino acids from hydrolyzed casein (St. Julian and Rose, 1932). The resulting material was supplemented with cystine, tryptophane, and histidine, and was incorporated in the diet at a level of 11.5 per cent (including the supplements). The rats which received this ration each gained at a rate of approximately 1 gram per day. The addition to the food of arginine, glutamic acid, aspartic acid, and proline failed to accelerate the increase in body weight. In view of the low level at which the hydrolysate was fed, it is difficult to interpret the results on any basis other than that the amino acids in question are not necessary dietary components.



Very recently, Wada (1930, 1933) has reported the isolation of a new amino acid, citrulline, from a tryptic digest of casein. The compound is said to have the following formula:



As will be observed, it is closely related to arginine. However, its presence in casein does not invalidate the experiments of St. Julian and Rose inasmuch as during acid hydrolysis citrulline is transformed into proline, and in this form is removed by repeated alcohol extractions. Final solution of the problem of the relation of the prolines, glutamic acids, arginine, and citrulline must await the use of a diet containing a synthetic mixture of amino acids known to be entirely devoid of the materials in question.

The present status of knowledge regarding the relation of the amino acids to nutrition is summarized in Table I. Of the twenty generally recognized protein components, the indispensable nature of only five has been positively established.

Two others, methionine and phenylalanine, appear to be capable of substituting for cystine and tyrosine respectively. The importance of seven is at the present time uncertain. Concerning the remaining six, available information does not warrant their classification with respect to maintenance and growth.

The bearing of the above facts upon the problem of the optimal protein intake is evident. In view of the well-known deficiencies of many proteins, notably those of plant origin, it would seem to be a safer procedure to consume more than the minimal amount necessary to maintain nitrogen equilibrium.

TABLE I.
TENTATIVE CLASSIFICATION OF AMINO ACIDS WITH RESPECT
TO THEIR NUTRITIVE IMPORTANCE.

Indispensable Amino Acids	Amino Acids which have not been definitely placed, but which appear to be dispensable	Amino Acids of unknown nutritive importance
Lysine Tryptophane Cystine (or methionine?) Histidine Tyrosine (or phenylalanine?)	Glycine Arginine Proline Hydroxyproline Aspartic acid Glutamic acid Hydroxyglutamic acid	Alanine Serine Valine Leucine Isoleucine Norleucine

Perhaps a suitable daily intake of protein for an adult of average size might be placed at 70 to 75 grams, or about 1 gram per kilogram of body weight per day. Such a quantity allows approximately 50 per cent surplus, as compared with the average Chittenden standard, in order to insure the presence of the essential amino acids in sufficient amounts.

The fundamental nature of investigations regarding the nutritive role of the individual amino acids need scarcely be emphasized. Obviously, adequate interpretation of the facts of protein metabolism, especially with respect to the growth process, will remain impossible until the importance of each amino acid has been determined. It appears that if further information of this sort is to be secured, one must either devise more adequate methods for the quantitative removal of single components of proteins, or resort to the use of synthetic mixtures

of highly purified amino acids. The latter alternative seemed to us to be the more promising, and has already yielded results of considerable interest.

Several attempts have been made in the past to feed mixtures of purified amino acids in place of proteins, but hitherto all such efforts have met with failure. Animals upon such rations have invariably declined in weight and finally died. For about three years experiments of this sort have been under way in the author's laboratory. In formulating the amino acid mixture we imitated the composition of casein in so far as available information permitted. Nineteen amino acids were used. Hydroxyglutamic acid was the only recognized protein component not incorporated in the food. That its absence was not significant was shown by supplementing the ration with a crude fraction of protein containing the dicarboxylic acids. The rats receiving the diets rapidly lost weight during the first 12 days, and then declined gradually or maintained weight to the end of the experiments (Rose, 1931). The results were interpreted as indicating that growth-promoting proteins contain at least one essential component other than the twenty known amino acids. In line with this conclusion, it was found that the addition to the diet of 5 per cent of casein, gliadin, or gelatin in place of an equivalent quantity of the amino acid mixture, was followed after 4 days by slow growth (Ellis and Rose, 1931). Evidently the supplements furnished something which was lacking in the amino acid mixture.

Inasmuch as casein proved to be more effective in stimulating growth than did either gliadin or gelatin, the former was employed in attempting to concentrate the active material (Windus, Catherwood, and Rose, 1931). For this purpose, hydrolyzed casein was fractionated into five groups of amino acids, namely, the less soluble ones, the dicarboxylic acids, the diamino acids, the alcohol-soluble material (proline), and the monoamino acids. The first four groups proved to be almost or completely devoid of growth-stimulating activity. On the other hand, the fraction of monoamino acids carried the unknown essential in much greater proportions than does whole casein. Indeed, by a second fractionation of part of the monoamino acids a material was obtained which induced normal growth when present in the food to the extent of 5 per cent.

The compound has been concentrated by several other methods of protein fractionation. By the use of the Town (1928) copper salts procedure, the growth essential is found to be associated with those amino acids whose copper salts are soluble both in water and in anhydrous methyl alcohol (Caldwell and Rose, unpublished data). In our best preparations, the chief contaminants of the unknown substance are believed to be valine and isoleucine. Incidentally, the compound is not identical with aminobutyric acid (Foreman, 1913; Abderhalden and Weil, 1913), the amino acids described by Schryver and his associates (1925-1927), norvaline (Abderhalden and Bahn, 1930), or any of the other protein disintegration products which have been mentioned from time to time in the literature. It appears, therefore, not to have been recognized heretofore.

Already we have learned many of its properties. These are being made use of in its further concentration. We are also conducting a study of its distribution in other proteins. We hope eventually to accomplish its isolation and identification. If we succeed, as we anticipate, we shall then be in position to determine with comparative ease which of the remaining amino acids are required for normal nutrition.

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A Case of Sex-Reversal in Man.

This curious story is the account of a well-known Danish painter, Einar Wegener, who started out in life as an outwardly normal male. He married, and lived an apparently normal life for some years. Gradually, however, he felt himself becoming psychologically a woman. No homosexual tendencies were evident. This psychological change continued to the point where, after being ridiculed by many physicians, he was at last taken in hand by an eminent Dresden specialist, who removed his sex organs, found rudimentary ovaries, implanted fresh ovaries, and made of the artist an essentially normal woman. The account is said to be strictly authentic and accurate. It is written in popular style, with no attempt at scientific analysis. A foreword by Dr. Norman Haire of London is more scientific, and verifies the essential facts of the book. Certain incidents strain the imagination somewhat, such as the change in the character of the handwriting from that of a man to that of a woman immediately following the first operation. Biologists and psychologists will find this unique story of considerable interest, as there would appear to be much debatable ground as to the interpretation of the initial changes in Wegener's makeup.—L. H. S.

Man into Woman. Edited by Niels Hoyer. 288 pp., 18 ill. New York, E. P. Dutton & Co., 1933. \$3.50.